Effects of organic mulching and fertilization treatments on root growth, nutrient partitioning and ectomycorrhizal populations of river birch.

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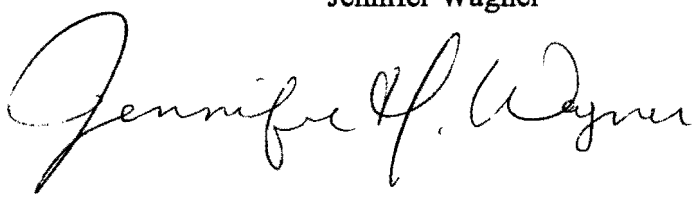

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Abstract. A relative lack of research on the effects of organic soil amendments in plant production and establishment is an obstacle to the rational use of these environmentally friendly resources. Mycorrhizas are symbiotic associations between some soil-borne fungi and the root systems of the majority of plants, and have been demonstrated to be beneficial to the fitness of the plant hosts. This study quantified and partially characterized populations of ectomycorrhizal fungi colonizing the roots of River birch (*Betula nigra)* trees grown in experimental plots under different treatments that included mulching with composted yard waste or shredded wood pallets, and N-P-K fertilization. It also evaluated root growth with respect to nitrogen cycling and nutrient partitioning within the plant. The various mulching and fertilization treatments, alone and in combination, were expected to elicit changes in ectomycorrhizal populations. Treatment appeared to affect the occurrence of morphotypes with a significantly higher diversity being found in the yard waste plots. Fertilization appeared to decrease total root mass/length, while mulching significantly increased colonization percentage. Carbon ratios of the mulches affected nitrogen cycling and thus nutrient allocation within the trees. Results of this study suggest that use of compost in tree establishment and maintenance may indirectly contribute to overall tree health by balancing available carbon and nitrogen stores. Thus, this study indicates that composted soil amendments may be a viable and superior alternative to mineral fertilization.

Introduction

Mycorrhizae

Whole microbial communities, performing the most essential functions of healthy soils, exist unseen beneath our feet. Fundamental components of these communities are mycorrhizal fungi which form specialized structures with the roots of some plants in a symbiotic relationship. Indeed, most plants have evolved with some sort of mycorrhizal association and are hence dependent on those specific associations for their very existence (Pfleger & Linderman 2000). The mycorrhizal fungi help to increase the nutrient absorbing capability of a plant's roots by colonizing the root tips, and extending into the surrounding soil (Alexopolous 1996). By this interaction, the root is afforded a surplus of nutrition beyond its hoarding capability. In this

fungus-plant relationship, the fungus is dependent on the plant for a source of carbon while the plant obtains several benefits from the colonizing fungus. Mycorrhizas provide several plant-assisting functions such as enhanced water absorption and nutrient assimilation, e.g. nitrogen and phorphorus, decomposition of inorganic and organic substrates, extending the life of feeder roots, and improving root disease resistance (Alexander 1981, Lakhanpal, et al. 2000, Marks & Kozlowski 1973).

Ectomycorrhizas

Several types of mycorrhizas have been described (Smith & Read 1997). This proposal deals with a large group of mycorrhizas, characterized by the fungus mostly inhabiting the outside of the root, termed "ectomycorrhizas". In this particular mycorrhizal 'system', hyphae grow strictly intercellularly within the root, and extend from the roots of the plant into the rhizosphere and the surrounding soil (Smith and Read 1997). To be classified as ectomycorrhizas, the mycorrhizas must have a Hartig Net, or the web-like result of intercellular hyphal growth between the outer epidermal cells of the root, and a mantle or sheath of fungal hyphae surrounding the outer surface of the root in either a loose or structured network (Smith and Read 1997).

One way to classify ectomycorrhizas themselves is by identifying their morphotypes, or morphological characteristics that tend to be congruent with fungal species identity on any given host. Key morphotyping features can include root tip branching pattern, tip shape, and emanating structures. Branching patterns of the mycorrhizal tips may range from simple to compound and clustered, while tip shapes and emanating structures such as cystidia may take on a variety of colorful shapes such as clubs or spikes. Other emanating structures include various

colored hyphal strands, i.e. threads of fungal cells, which then take on a variety of frequencies and patterns depending on the morphotype.

Ectomycorrhizas are formed by fungi within the taxonomic orders of Basidiomycetes, Ascomycetes, and the genus Endogone within the Zygomycetous order (Smith and Read, 1997). Ectomycorrhizas are most commonly found growing on the roots of gymnosperms, *Pinus* in particular, although angiosperm trees, e.g. in the families Fagaceae, Betulaceae, Salicaceae, and Ulmaceae, shrubs, and herbs are also associated (Lee & Alexander 1981, Marks & Kozlowski 1973). Morphotype frequency (i.e. how often they appear with respect to other morphotypes) and abundance (i.e. how plentiful they are), help to give clues about the importance of these mycorrhizas. The observation of ectomycorrhizal growth on a vast majority of temperate and boreal forest trees, and a healthy tree's importance to the vitality of an ecosystem, make research on this symbiosis particularly significant to the scientific community.

Compost

Aerobic composting is a process in which organic materials are converted into a more stable form for use as a soil amendment. In the aerobic formation of compost, various saprobic microorganisms, i.e. fungi, bacteria, protozoa, and actinomycetes combine oxygen with carbon from dead or decaying organic matter to complete various life processes. A majority of the carbon is respired as carbon dioxide while a portion of the rest is combined with nitrogen in living cells. Hence, an optimal C:N ratio for a compost mix is typically considered to be 30:1 (Epstein 1998, Rynk 1998). Aerobic decomposition is characterized by fluctuating temperatures that favor different microbial groups in digesting the materials comprised within the compost pile. During these extremes, fungi and actinomycetes prevail in mesophilic temperature

conditions ranging from 50-115 °F, while bacteria colonize the material in thermophilic conditions of 115-160 °F (Biocycle eds. 1989). A properly composted product will have reached high enough temperatures to render the material devoid of harmful pathogens and weed seeds while sustaining a properly balanced C:N ratio. In a sense, this process allows for the accelerated imitation of natural processes that create healthy soil.

The overall function of compost in plant growth appears to be that it provides a stable environment for beneficial microorganisms while improving soil tilth and structure. In this environment, a low C:N ratio allows for the timely release of nutrients that supply the growing microbial community as well as the plant with a sustainable level of carbon and nitrogen. Therefore, compost can be an optimal substrate for growing plants as it benefits both the plant, its microbial partners, and the environment.

Nitrogen Cycling and Resource Partitioning

The basic theory of matter/nutrient partitioning in plants states that carbon in the form of sugars travels from leaves to shoots and roots, and nitrogen flows from the roots to nourish leaves. Resource partitioning in plants is considered adaptive, and allocation is dependent mostly on nitrogen concentration within the whole plant (Bradshaw 1965, Hirose 1987, Ingstad & Agran 1991). Resource allocation patterns are most noted when some nutrient, e.g. carbon or nitrogen, are limited to the plant.

Soil nitrogen has two very important effects on mycorrhizal associations as well as plant growth and resource allocation in relation to this study. Overall nitrogen levels within the whole plant depend to a large degree on soil resources of inorganic molecules as well as water. Increasing nitrogen availability in the soil will increase concentrations of nitrogen within the

whole plant (Hirose 1987). Several sources document that nutrient poor soil environments, including soil shortages of both carbon and nitrogen, tend to favor root growth (Bloom *et al.* 1985, Chapin 1980, Ericsson 1995, Epstein 1972, Hirose 1987, Ingstad & Agren 1991). Probable explanation for this is that roots try to maximize their absorption by increasing surface to volume ratio during times of nutrient stress (Epstein 1972). In the wild, plants make use of mycorrhizal associations to promote high root:shoot ratios in infertile soils (Chapin 1980, Hirose 1987, Ingstad & Agren 1991). It must be noted that resource partitioning in plants as well as mycorrhizal relationships is largely species/environment-specific (Bloom *et al.* 1985, Chapin & Mooney 1985, Ingstad & Agren 1991).

Several sources document that ectomycorrhizas in particular are much more capable of absorbing reduced forms of nitrogen such as NH_4 or a combined form of NH_4 and organic nitrogen than NO_3, and that high fertilization rates may actually inhibit mycorrhizal formation (Nye & Tinker 1977, Raven, Wollenweber & Handley 1992). As a result, ectomycorrhizal development tends to be most prolific where both NH_4 is the dominant form of nitrogen, and soil pH, among other factors, slows nitrogen mineralization (Alexander 1981).

Problem Identification and Justification

Compost and ectomycorrhizas have unique abilities to improve the soil and a plant's growing conditions. Therefore, information and research on compost and its function in the landscape is valuable. However, there is a lack of documented research on fertility regimens and ectomycorrhizal associations in the urban landscape. The combined analysis of ectomycorrhizal morphotypes, soil fertility, and resource partitioning in the plant can shed new light on the relatively unknown realm of the urban landscape ecosystem.

Birch trees in particular could be interesting to the urban environment for their ability to tolerate wet, poorly drained, acidic soils with nearly toxic concentrations of aluminum, calcium and magnesium (Cribben & Irwin 1974). This study attempted to not only quantify the various forms of birch mycorrhizas, but went a step further to evaluate the role of mulching and fertility treatments on both the proliferation of these beneficial symbionts and root growth on river birch. Analysis of soil fertility in relation to organic matter and nitrogen cycling was also done in the context of an allied investigation (Lloyd 2001).

Hypothesis and Objectives

Hypothesis: Given its low C:N ratio, composted yard waste should optimize and balance soil health and root/shoot growth and resource partitioning, thus decreasing the trees' need to enlarge root systems and support mycorrhizas.

To test this hypothesis we pursued the following objectives:

1) Isolate ectomycorrhizas colonizing the roots of *Betula nigra* grown in soil fertilized and/or mulched with composted yard waste or ground wood pallets and determine their morphotype diversity and frequency.

2) Quantify root growth and evaluate nitrogen cycling to rate which organic mulch, if any, improved root growth and ectomycorrhizal diversity, frequency and/or abundance, and how mineral fertilization modulated this response.

Materials and Methods

This experiment was integrated into a project conducted in 1998-1999 in Wooster, Ohio at the Ohio Agricultural Research and Development Center, by Herms' group (OSU/OARDC Entomology). Several fertilization and soil amendment regimens were tested on the growth of two-year-old river birch *(Betula nigra)* (Lloyd *et. al*, 2000). Statistical design of the project was arranged with type of soil amendment as fixed variables: shredded wood pallets, composted yard waste, or basic field soil. Half of the blocks were fertilized with a 18:5:4 N-P-K fertilizer twice per year for two years at a rate of 150 kg N ha^{-1} yr^{-1} (Lloyd 2001). Mulches were surface-applied 5 cm thick at two different times during the course of the experiment (Lloyd 2001). The two experimental blocks were composed of 24 individually separated, 1 m^3, plastic lined cells per block each containing 1 river birch, 1 yew and 1 rhododendron. Only the birch trees were evaluated in this study.

This study reflects the analysis of 5 cm diameter by 10 cm deep soil cores taken approximately 6" from the base of each tree after removal of the mulch layers (Lloyd 2001). Cores were stored in rigid plastic liners at –20°C from November 2000 until September 2001, when cores were analyzed. Cores were thawed in 75% ethanol to preserve fungal DNA for future analysis. After coming to room temperature, the roots were separated from the slurry by pouring the beaker's contents through a fine-meshed screen. A magnifying glass with light was used to separate fine roots from soil particles. Fine roots were placed into a fresh beaker of 75% ethanol, sub-sampled and placed in an 8.5 cm diameter plate filled with 75% ethanol. Dead and large root fragments and debris were removed from the sample prior to quantification.

Existing quantification techniques published in mycorrhizal manuals were used. In particular, we employed the gridline intersection method and a suite of mycorrhizal characters

listed in Goodman *et al.* (1995). The gridline intersection method (Tennant 1974) was employed by observing a 0.5" grid placed beneath the petri dish under magnification, and colonization percentages were calculated from measurements. Furthermore, obvious morphotypes were separated into different petri plates for subsequent evaluation and determination of frequency and abundance. Individual mycorrhizal root tip and hyphal strand lengths and widths were obtained using a small swatch of 1 mm fiberglass insect screening placed in the dissecting microscope's field. Samples of mycorrhizal root tips were cross-sectioned using a scalpel, stained with Toluidine Blue in phosphate buffer, and Meltzer's Reagent, then mounted on slides and observed at 40x to clarify identification characteristics as recommended for North American ectomycorrhizal fungi by Goodman *et al.* (1995), and Agerer (1991).

Statistical Analysis

All data for the soil core parameters e.g. amendment type and fertilization regimen as fixed factors, and measurements of dependent variables, e.g. number of morphotypes, total root length (includes mycorrhizas), total mycorrhizal root length, percentage colonization, and mean dry mycorrhizal mass were compiled and tested using SPSS 10 software for Windows or SAS for Windows to determine correlations and treatment differences using analysis of variance (ANOVA). Root measurements from soil cores were correlated with nitrogen cycling parameters, which included measurements of soil organic matter content, microbial respiration rate, nitrogen mineralization rate, and plant usable nitrogen (Lloyd 2001), recorded on numerous dates during 1999-2000 from the Wooster study.

Results

Nutrient Cycling

Results of the Wooster study showed that the mulches, on average, increased soil organic matter (fig. 1), but only consistently each year with composted yard waste which had the highest overall organic matter content during the study (Lloyd 2001). Microbial respiration was also significantly increased by both mulches (fig. 2) (Lloyd 2001). Fertilization affected neither soil organic matter percentage nor microbial respiration (Lloyd 2001). Mulching with yard waste significantly increased plant available nitrogen over wood pallets and bare soil by 15-60% during 1999 and by 20% during half of 2000 where the wood pallet treatment also increased available nitrogen (fig. 3) (Lloyd 2001). Fertilization only significantly affected plant available nitrogen by 24% in April of 2000 for wood pallets and by 17% in August of 2000 for yard waste (Lloyd 2001).

Plant Resource Partitioning

The ratio of dry root mass of river birch to trunk diameter decreased non-significantly with fertilizer application, and was lowest for yard waste and highest for wood pallets respectively within each fertility treatment (table 1) (Lloyd 2001). Fertilization significantly decreased total root length by 28% amongst all the treatments ($P = 0.03$) (fig. 4). The decrease was most marked in the wood pallet treatments where root length decreased by 47% (fig. 5). Both mycorrhizal length (fig. 6) and total root length (fig. 7) increased as total soil nitrogen increased ($P = 0.007$ and $P = 0.048$, respectively). Percent mycorrhizal roots decreased as total soil nitrogen increased ($P = 0.026$) (fig. 8). There was a strong positive relationship between soil ammonium and root length ($P = 0.029$ (fig. 9).

The compost amendment significantly increased the number of morphotypes ($P = 0.01$) (fig. 10), and significantly decreased percent of mycorrhizal roots amongst all mulches ($P = 0.02$) (fig. 11). The two morphotypes found were labeled A and B respectively (fig.s 12 and 13). Morphotype A had a highly branched system with straight to bent tips that were yellow or brown with age, and had a felty texture. Hyphal strands were not common in this morphotype. Morphotype B had a simple system of tips that were straight to club-shaped, light to dark yellow color, and had a cottony texture. Hyphal strands were light yellow and common. Most mycorrhizas were of morphotype A.

Discussion

Results of this experiment fall in line with theories of mineral plasticity in plants. Fertility treatments significantly affected root growth, number of ectomycorrhizal morphotypes, and mycorrhizal percentages. Fertilization had little effect on soil organic matter or microbial respiration throughout the study (Lloyd 2001). However, as consistent with theories of resource partitioning, fertilization decreased root growth and mycorrhizal colonization percentages. Fertilization especially decreased total root length in plots with high C:N ratios like the wood pallet plots. Measurements of root:shoot ratios, though merely trends, appear to support the notion that nutrient partitioning was taking place in the birch trees. Excess nitrogen probably relaxed competition between plant roots and carbon-loaded microbes for nitrogen requirements. As a supply of available nitrogen was readily applied, more nitrogen was available to be sequestered to above ground shoots and leaves so that, overall, root length decreased. This result is consistent with the general theory of nutrient plasticity in plants placed forth and advocated by Bradshaw (1960), Chapin (1980), and many others.

In general, mulching added carbon and nitrogen, microbial fuel/energy, to the soil. These observations are reinforced by correlating data for increased microbial respiration rates in the plots which shows that soil microbiota were more active in mulched plots. Constant values for increased organic matter percentages for the yard waste plots, and inconsistent values for wood pallet plots, may indicate that a composted material slowly breaks down its nutrient components over a period of time. Yard waste plots also produced the lowest values for root length and density, further insinuating that resource partitioning may have been more balanced within the plant due to the low C:N ratio of the composted yard waste mulch (refer to fig. 5 and table 1). This is also supported by the fact that only mulch with low C:N ratio, i.e. composted yard waste increased nitrogen availability (fig. 3). Figure 4 probably most accurately reflects the abundance of root growth in the unfertilized wood pallet mulch plots where available soil nitrogen was lowest overall (fig. 3).

Root length increased with soil ammonium concentrations as well as with total soil nitrogen (includes N tied up in microbial biomass), and mycorrhizal length also increased with total soil N (fig.s 6, 7, and 9). This may support the theory that ectomycorrhizas have a preference for reduced forms of nitrogen. According to Marks and Kozlowski (1973) ectomycorrhizas are unable to use nitrate as a nitrogen source because they lack nitrate reductase necessary to mineralize the NO_3. Raven (1992) and Raven et al. (1985) suggested that NH_4 nitrogen causes a faster growth rate in certain plants and algae than does NO_3 nitrogen, and also takes less energy to manipulate to produce final plant-needed nitrogen-containing compounds. This hypothesis could also be supported by the work of Zhang *et al* (1999) who showed that nitrate nitrogen might actually act as a chemical signal for root branching and cell elongation instead of cell proliferation, and that NO_3 may also inhibit root branching in excessively high

amounts in *Arabadopsis*. This study was based on the premise that nitrate is a chemical signaling molecule, and not a nutrient complex.

Conversely, as total soil nitrogen increased, the percent of mycorrhizal roots decreased. As total root length increased, less root was being colonized by mycorrhizal fungi. Ectomycorrhizas need nitrogen to perform specific life functions, but increasing the pool of plant available resources, including nitrogen, allows the plant to harvest its own nitrogen from the soil thereby lessening the need to utilize mycorrhizas (Alexander 1983, Ruehle & Wells 1984, Beckjord *et al.* 1984).

Most measurable effects of amendment were seen in yard waste plots. Compost plots showed the highest diversity of ectomycorrhizal morphotypes (fig. 10) and the lowest percent mycorrhizal colonization. The compost, having a low C:N ratio and thus supplying the soil with ample usable nitrogen over time probably relaxed plant/microbe competition and reduced the plant's need for maximizing its root zone with mycorrhizal associations (Alexander 1983, Beckjord *et al.* 1984, Marks & Kozlowski 1973, Ruehle & Wells 1984). In contrast, the higher amount of organic matter found in the composted yard waste may have stimulated the diversity of soil microbiota including ectomycorrhizal morphotypes (Lloyd 2001).

The aforementioned discussion on soil nutrient parameters and plant growth aligns with the work of Gleeson (1992), Ericsson (1995), Bloom *et al.* (1985), and others which elaborates on the cyclic nature of resource partitioning in plants which is dependent on the overall driving need to survive. Gleeson (1992) especially elaborates on the variation in nitrogen supply and its varying effect on tissue nitrogen and root:shoot allocation. Bloom *et al.* (1985) relates resource partitioning in plants to fiscal economics interjecting that resource allocation occurs, presumably, because evolution has allowed plants to adapt both long and short-term.

Fig. 1 Soil organic matter expressed as a percentage of total soil sample. Numbers represent averages of data collected at various times throughout the 2 years of the study (Lloyd 2001). Con = control soil (no mulching); WP = wood pallets; YW = composted yard waste.

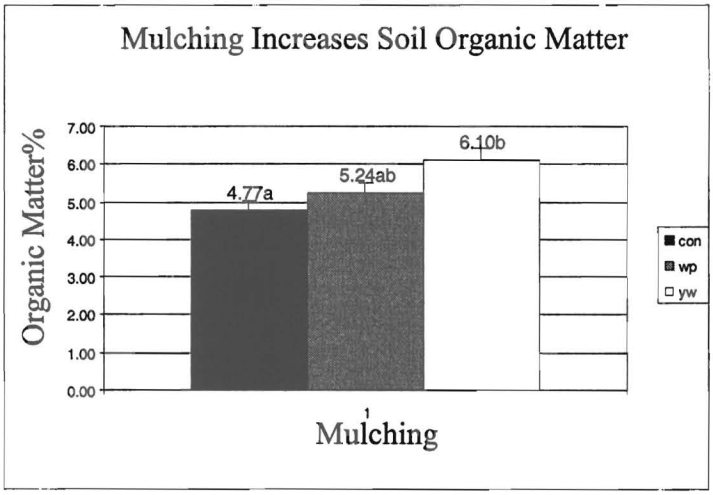


Fig. 2 Microbial respiration expressed as mg CO2 / g soil / day for control plots and mulched plots. Numbers represent averages of respiration measurements taken at various times throughout the stu (Lloyd 2001)..
Con = control soil (no mulching); WP = wood pallets; YW = composted yard waste.

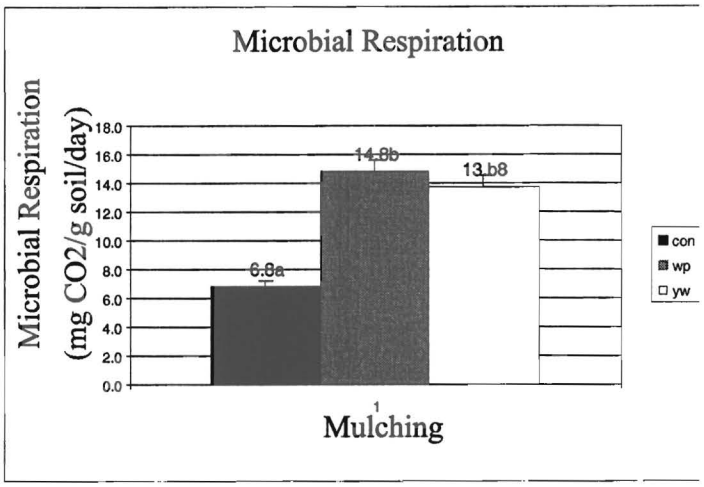


Fig. 3 Plant available nitrogen expressed as total available nitrogen in the form of $NO_3 + NH_4$ + dissolved organic nitrogen (DON) in ppm (Lloyd 2001). Numbers represent averages of samples taken throughout the study. Con = control soil (no mulching); WP = wood pallets; YW = composted yard waste.

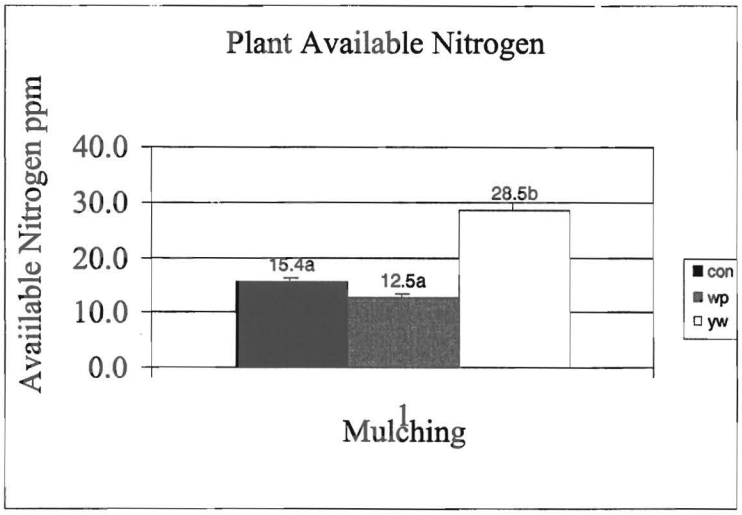


Table 1. Non-significant data show that ratio of total root mass (mg) to trunk diameter (inches) changes with fertility. WP = wood pallets; YW = composted yard waste.

	Fertilized		Non Fert
Control	8.3		15.2
WP	10.7		16.4
YW	7.0		14.5

Fig. 4 Overall effect of fertilization on root length for all cores.

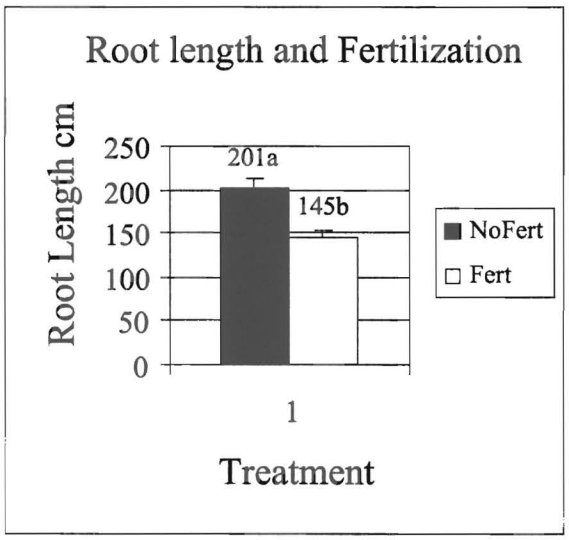


Fig. 5 Effect of fertilization and amendment on root length

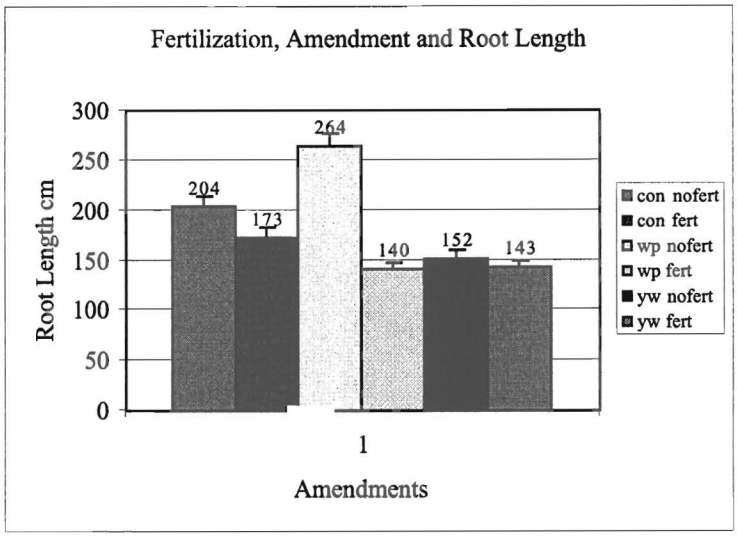


Fig. 6 Total soil nitrogen as it relates to total root length. Total soil nitrogen refers to ammonium, nitrate, dissolved organic nitrogen, and nitrogen tied up in microbial mass (Lloyd 2001).

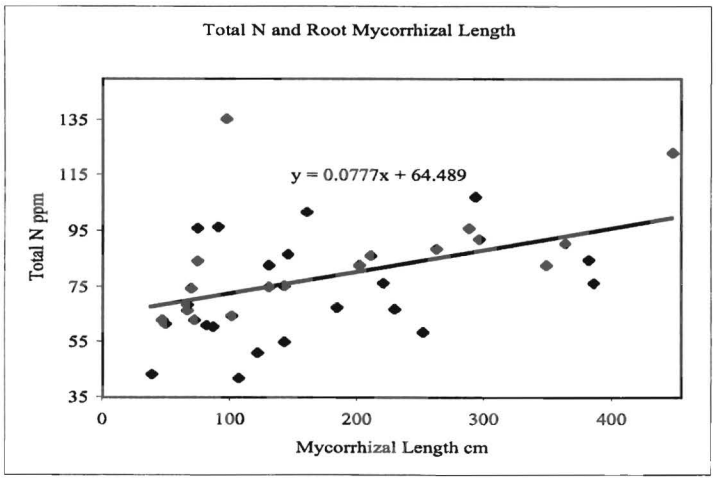


Fig. 7 Total soil nitrogen (ppm) as it relates to total mycorrhizal length (cm) (Lloyd 2001).

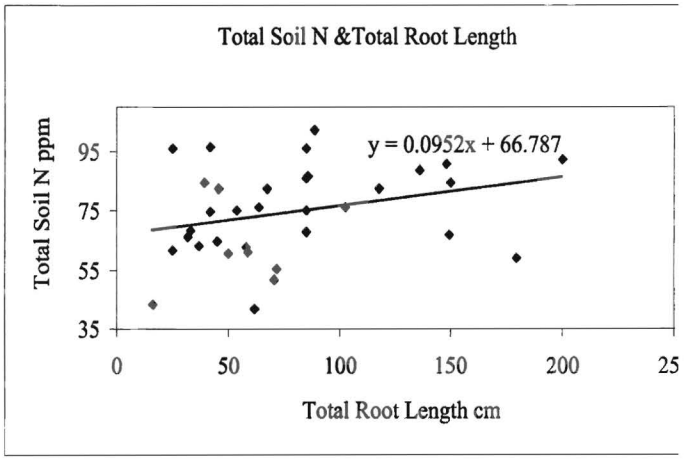


Fig 8 Relationship between total soil N and percentage of birch roots that were mycorrhizal (Lloyd 2001.

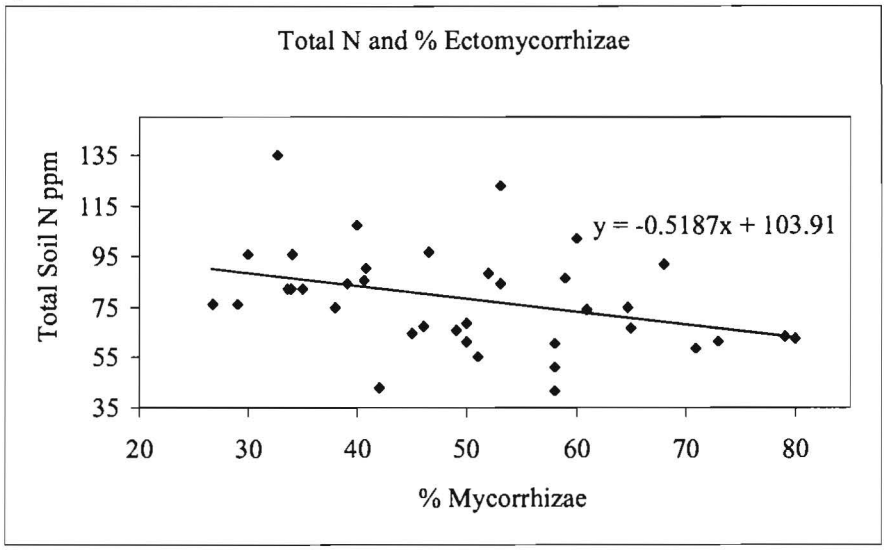


Fig. 9 Relationship between soil ammonium (ppm) and total root length (cm) (Lloyd 2001).

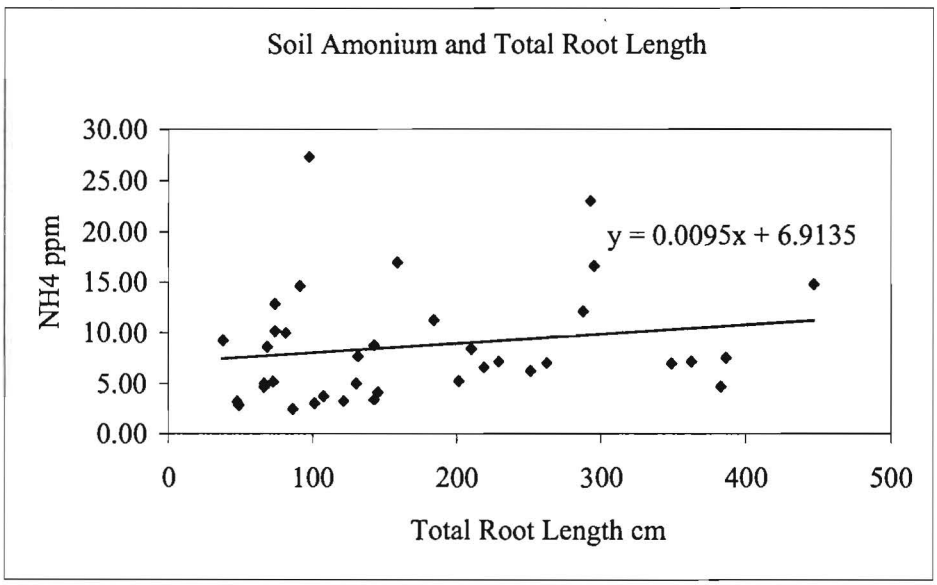


Fig 10 Effects of mulching treatment on mycorrhizal diversity. The average number of morphotypes/core was significantly higher in the composted yard waste treatment.

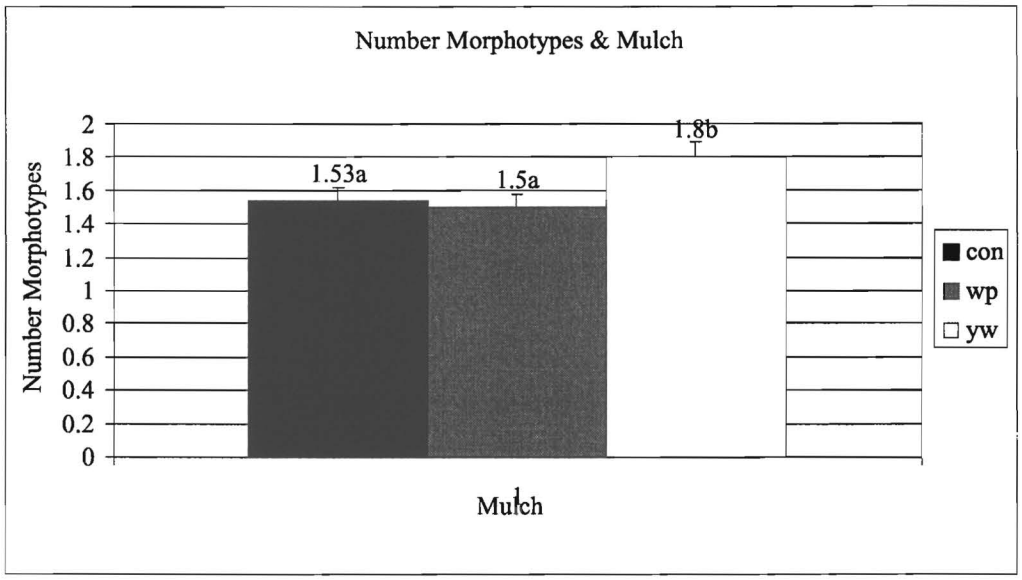


Fig. 11 Effects of soil amendment on the percentage of root system colonized by mycorrhizal fungi. The compost amendment significantly decreased colonization percentage with respect to the control soil.

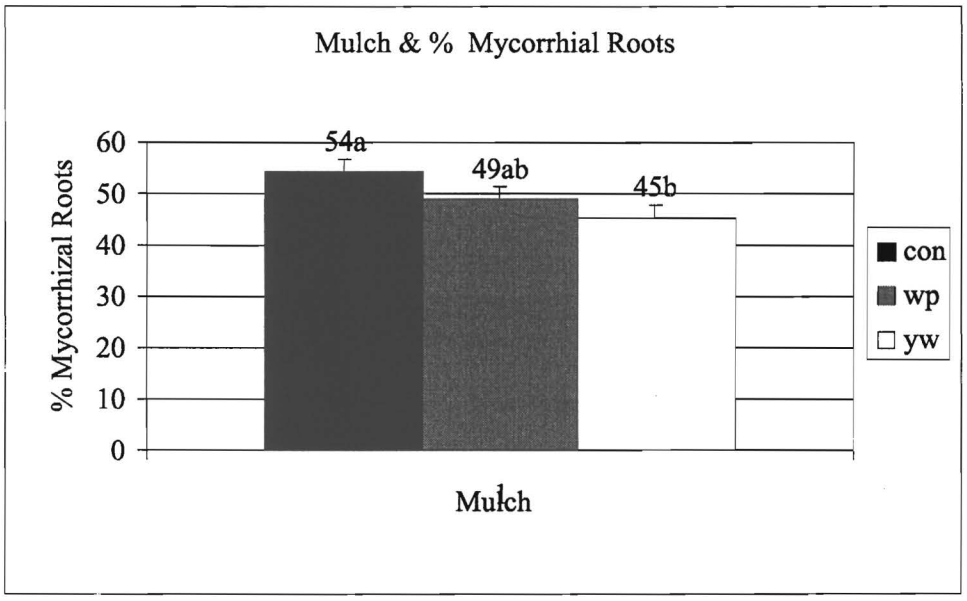


Fig. 12 Ectomycorrhizal type morphotype A. System root tip and whole system mass.

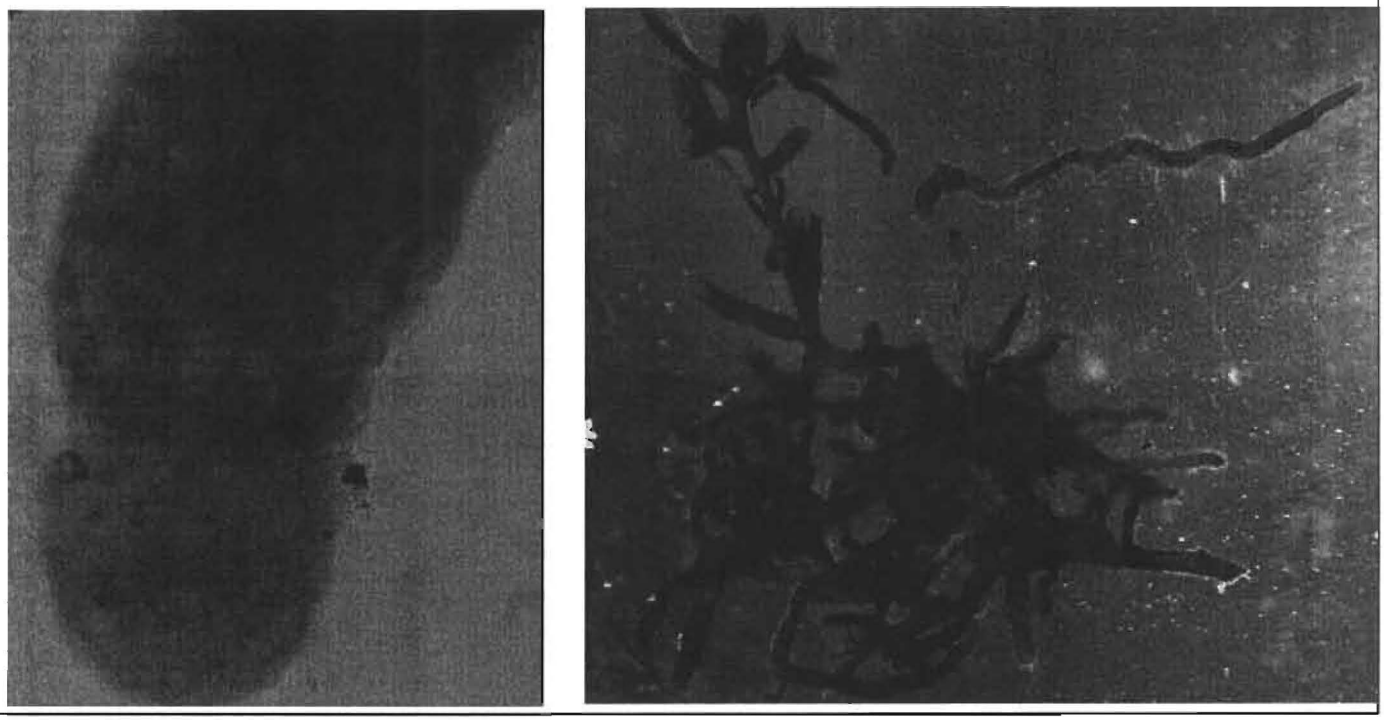
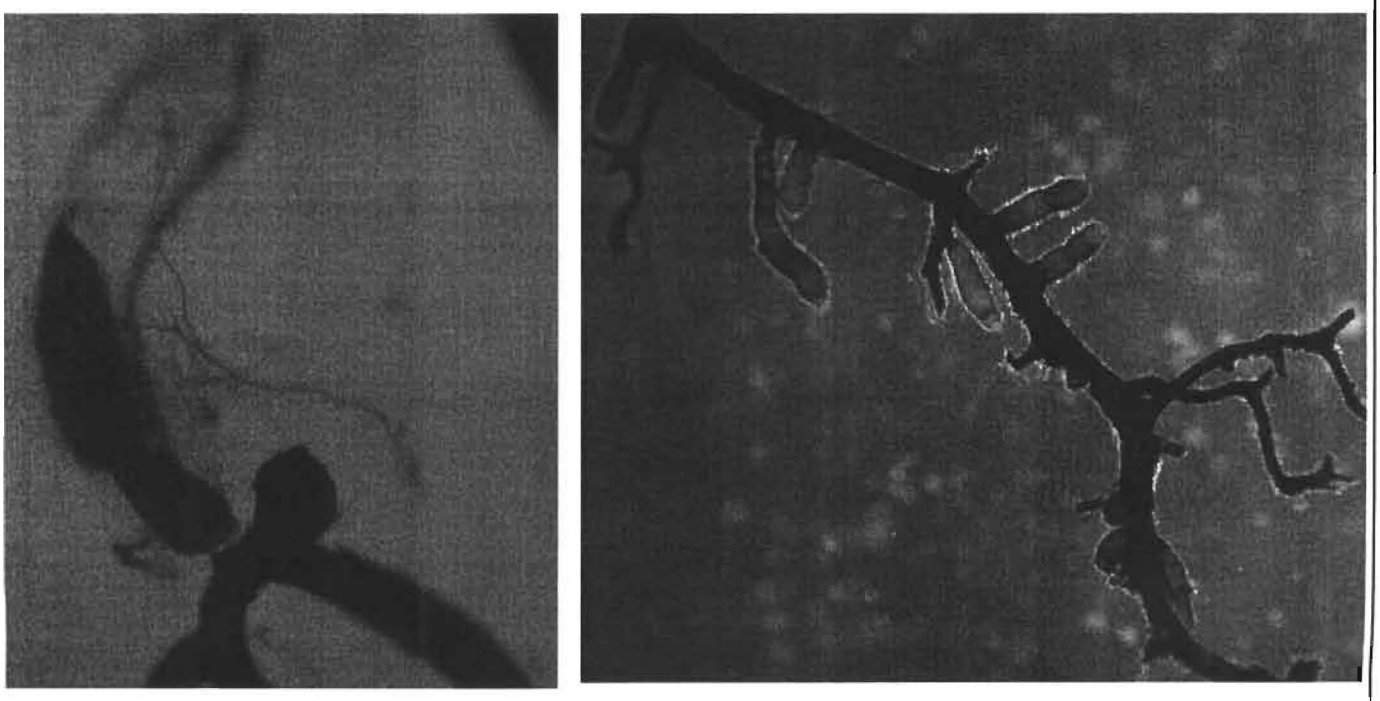


Fig. 13 Ectomycorrhizal morphotype B. System root tip and whole system mass.



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